Electron-microscopic findings after transmyocardial laser revascularization in an acute ischemic pig model

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Abstract

Objective: The clinical benefit in terms of angina reduction after transmyocardial laser revascularization (TMLR) in patients with diffuse coronary artery disease who are not candidates for conventional procedures has been proved. The exact mechanisms of TMLR however, are still unknown. The aim of this study was to investigate the cellular changes in relation to intramyocardial partial oxygen pressure (pO₂) after TMLR in a model of acute ischemia in pigs by electron microscopical methods (TEM).

Methods: Seven pigs were included in this study (five animals with acute myocardial ischemia and additional TMLR and two animals with acute myocardial ischemia and without TMLR for control). Acute ischemia was induced by ligation of diagonal branches of the left anterior descending artery (LAD). Intramyocardial partial oxygen pressure was measured before induction of ischemia and thereafter continuously for up to 6 h in all animals. Biopsies of all animals were taken before induction of ischemia and thereafter at 30 min, 3 and 6 h. Analysis of the myocardial ultrastructure was focused on mitochondria, cell nucleus, T-tubules and myofibrils.

Results: Ultrastructural changes were seen in all animals. At 6 h after induction of ischemia, mitochondria showed a destruction of the internal as well as the external membrane and of the cristae. The nuclei showed margination of the chromatin. Myofibrils were characterized by ruptures in the Z-stripes. Lipid droplets as an indicator of ischemia could be identified. pO₂ between 40 and 80 mmHg before intervention decreased down to 0–2 mmHg within the first 9 min after diagonal branch ligation and did not increase even after TMLR. Conclusions: In this acute ischemic model using pigs, TEM evaluation following TMLR proves irreversible changes of the myocardial ultrastructure. Furthermore, TMLR was not able to increase ischemically induced decrease of pO₂. These data provide some evidence that TMLR thus, may not be able to ameliorate acute ischemia at least in the pig model. Further investigations are needed to investigate the effect of TMLR in chronic myocardial ischemia. © 1997 Elsevier Science B.V. All rights reserved.

Keywords: Coronary artery disease; TMLR; Myocardial ultrastructure; Partial oxygen pressure

1. Introduction

Transmyocardial laser revascularization (TMLR) is a new alternative therapeutic method in the treatment of severe diffuse ischemic heart disease [1–3]. Symptomatic patients not treatable with conventional methods, such as coronary revascularization or angioplasty, were considered for TMLR [4,5]. The first clinical results indicated a significant improvement in the clinical symptoms of these patients [5–7]. The exact mechanism of TMLR therapy is still unknown, and furthermore, it is controversially discussed as to how far acute ischemia can be prevented by this form of
therapy [8–11]. Authors have concentrated their investigations with TMLR on acute ischemic models, mainly analyzing myocardial function, determining infarct size, and observing myocardial perfusion, but not on investigating myocardial ultrastructural changes in relation to intramyocardial p\textsubscript{O2} measurement in a pig model, which is the aim of this study.

2. Material and method

Seven pigs with induced acute myocardial ischemia were included in this study. Five pigs were treated with TMLR and two pigs were used as controls. This project was approved by the Ministry of Environmental Affairs, and the Ministry of Nature and Agriculture by the country Schleswig-Holstein on the 27th November 1996. The animal environment and care was maintained to the conventional European guidelines on the care of a veterinarian.

2.1. Anaesthesia and operative technique

The animals were premedicated with 250 mg ketamine subcutaneously (s.c), rompune 2% s.c., 15 mg midazolame s.c. and 1 mg atropine s.c. The anesthesia was intravenously (i.v.) introduced as a bolus application of 50 mg ketamine and 50 mg disoprivane. Continuation of anesthesia was achieved with a mixture of isoflurane 0.8%, ketamine (100 mg/h. i.v.), disoprivane (500 mg/h i.v.) and pancuronium (8 mg/h i.v.). Ventilation was performed with a Sulla respirator (Dräger, Lübeck, Germany) and the ventilation parameters were set at: FiO\textsubscript{2} = 1.0, and flows of 6 l/min (ideal pCO\textsubscript{2} = 38–42 mmHg). Hemodynamic parameters measured were arterial blood pressure in the femoral artery, central venous pressure in the jugular vein and ECG. Crystalloid solutions were used for volume replacement. The animals hearts were exposed by median sternotomy and pericardium opening. Amiodarone i.v. as a rapid infusion of 300 mg was applied profilactically as antiarrhythmic treatment before manipulation of the heart. Under anesthesia, animals were sacrificed 6 h after induction of ischemia by injecting 50 ml potassium chloride.

2.2. Induction of ischemia and TMLR

An acute ischemia was induced by ligating the different diagonal branches of the LAD. Ischemia was defined as a drop of p\textsubscript{O2} down to values between 0 and 2 mmHg, which occurred within the first 9 min after ligation in all pigs. At this time, TMLR treatment was applied. Macroscopically ischemic areas impressed as a change of the myocardial colour into violet. After induction of ischemia, TMLR was performed in five animals with four to eight channels at a density of approximately one channel per cm\textsuperscript{2} in the area of macroscopically demarked ischemic myocardium. A high power, 800 W CO\textsubscript{2} laser system (PLC Medical Systems, USA) was used. The energy was set at 40 J with an impulse rate of 50 ms. TMLR was performed on the beating heart triggered by ECG in the end diastolic phase. An echocardiogram (Hewlett Packard Sonos 2500) was used to determine if the shots succeeded through the myocardial wall by detecting air vaporization bubbles in the left ventricle.

2.3. Electronmicroscopic evaluations

By means of a punch (Radioplast, Uppsala, Sweden), myocardial tissue biopsies were taken at the following time intervals:
1. In all animals before induction of acute ischemia to evaluate the non ischemic vital myocardium. Biopsies were taken from the diagonal branch area of the LAD.
2. In five animals with acute ischemia and with additional TMLR at 30 min, 3 and 6 h after induction of ischemia initiation to evaluate the effect of TMLR on ischemic tissue.
3. In two animals with acute ischemia and without TMLR at 30 min, 3 and 6 h after induction of ischemia to determine the induced ultrastructural changes. Biopsies were taken from the area of macroscopically demarcation of ischemia.

Biopsies of ~ 1 cm (length) and 1 mm (diameter) were taken from the area between the TMLR channels. To avoid transmyocardial biopsies and thus, interference with the TMLR procedure, only one biopsy per pig was, at the defined time scale, taken. Punches were performed at a 45° angle about tangential direction into the mean position of the myocardium.

All myocardial tissue biopsies were cut into five pieces to analyse different myocardial areas. Fixation was done in Monti-Graziadei solution (2% glutaraldehyde and 0.6% paraformaldehyde). Postfixation was then continued with 2% osmiumtetroxyde for 2 h. After dehydration in rinsing alcohol series (30–100%) and embedding in araldit, contrasting was completed with uranylacetate (30 min at 40°C) and ledicitrate (6 min at 20°C). Ultrathin slices were performed with an Ultracut E (Reichert and Jung, Germany), and evaluated with a Philips 400T electronmicroscope with special focus on nucleus, mitochondria, T-tubules and myofibrils. Ultrastructural changes of the myocardium in the animals with ischemia and TMLR were compared to the TEM findings in the animals with ischemia and no additional TMLR.
Fig. 1. The myocardial ultrastructure of the vital not ischemic myocardium. Nucleus: It shows several deep furrow-like notches and a clear nucleus. The chromatin granule is homogenically spread, and somewhat more densely accumulated along the nucleus membrane. Mitochondria: They are also densely accumulated around the nucleus. Between mitochondria and the nucleus membrane-like structures, fine filaments and glycogen granules are accumulated. Between the myofibrils, the mitochondria are lined in one row. T-tubule: The lumina of the T-tubules contain basal-lamina material. Dark stripes along the T-tubules enable the connection to the endoplasmatic reticulum. Myofibrils: They appear contracted.

Fig. 2. The myocardial ultrastructure after 30 min of ischemia. Nucleus: Densely accumulated chromatin granules lined along the nucleus (margination) and as clots in the mid-nucleus. The nucleus is light and of fine structure. Mitochondria: Many mitochondria show destroyed cristae. Membrane-like structures, fine filaments and glycogen granule vanished. Some mitochondria contact the nucleus. The T-tubule and myofibrils show no significant structural changes.

Fig. 3. The myocardial ultrastructure 6 h after ischemia. Nucleus: Indicates a strong clotting and a clear margination of the chromatin. Mitochondria: They lie isolated around the nucleus in a niche. In the mitochondria lie several larger electron density granules. T-tubule: They show a vague basal lamina. Myofibrils: They seem relaxed and partly in the height of the Z-stripes ruptured and vanished. Between them lies a larger, round homogenous structure, that is enclosed by a membrane-like structure. This was identified as lipid droplets.
2.4. $P_{\text{t}}O_2$ measurement

Oxygen measuring probes (GMS, Mielkendorf, Germany) were positioned for measurement of $P_{\text{t}}O_2$ in the diagonal area of the LAD between the laser channels to keep equal distances from each channel to the probe. The validity of this method has been described by other authors [12]. In brief, this methods applies the principle of a polarographic probe with a gold cathode and a silver anode placed in a chamber filled with buffered electrolytes, into whom oxygen diffuses through a diffusion membrane resulting in the occurrence of a circuit. Measuring probes were connected to a computer device for the continuous measurement and documentation of regional tissue oxygen tension.

3. Results

3.1. Electronmicroscopic findings

The ultrastructure in the vital pig myocardium is demonstrated in Fig. 1, with a normal appearance of the cell organs. Myocardial ultrastructural changes following acute ischemia taken at 30 min, 3 and 6 h after induction of ischemia only shows slight differences. The extremities of these three stadiums are depicted in Figs. 2 and 3. They show the typical ultrastructural changes following acute ischemia (for detailed description see legends Figs. 2 and 3). After induction of acute ischemia and additional TMLR, the same ultrastructural changes were observed as in acute ischemia without TMLR (Figs. 4 and 5). Typically, ischemic caused changes of mitochondria and the cell nucleus and myofibrils occured and could be seen in all cell organelles 6 h after TMLR (for further details see legends Figs. 4 and 5).

3.2. Course of $P_{\text{t}}O_2$

$P_{\text{t}}O_2$ before intervention was 40–80 mmHg in all animals. After induction of acute ischemia, $P_{\text{t}}O_2$ decreased down to 0–2 mmHg within the first 9 min in all pigs. There was no increase of $P_{\text{t}}O_2$ observed up to 6 h after induction of ischemia in animals with and without TMLR.

4. Discussion

The reports about clinical application of TMLR show an improvement of symptoms in patients with diffuse ischemic coronary artery disease whereby the exact mechanism of TMLR effect is still speculated [5–7]. The principle of TMLR in terms of myocardial perfusion via intramyocardial channels from the left ventricular cavity into the myocardium, as in amphibians, is more and more questioned [13]. Thus, other mechanisms seem to cause the TMLR effect. The findings of histologic evaluation of laser channels in patients that had died are also controversial. Mirhoseini and colleagues [1] found still open laser channels even after months, whereas other authors observed all channels to be closed in their histological examinations [14,15]. TMLR, leading to an induction of angioneogenesis is a well discussed possible mechanism of this method. Fisher and colleagues described neocapillarization in a comparison of two different laser systems [16]. However, there is still uncertainty as to whether TMLR therapy can prevent acute ischemia. Yano and colleagues [17] found that non-transmural lasering from the endocardial side of the left ventricle could protect the myocardial function from the induction of ischemia. Lasering was performed here before induction of acute ischemia, in an animal experiment with dogs.

Also, Whittaker and colleagues [18] could prove that infarct size could be reduced when TMLR was performed before induction of ischemia, but in their study, lasering was performed 2 months before acute ischemic initiation and their investigations were carried out in rat hearts. Kadipasaoguloa and colleagues [8] also carried out experiments on a dog model, on the effect of TMLR after induction of acute ischemia. They found that 3 months after TMLR, left ventricular function in the laser group compared to controls was significantly better. These findings are contrary to the results of another study by Whittaker and coworkers [9] who did not observe any immediate benefit of TMLR on acute ischemic myocardium. In their study, TMLR was performed 30 min after LAD closure in a dog model, whereby lasering could not reduce infarct size.

All authors investigated left ventricular function, different infarct sizes, and myocardial perfusion distribution, mainly to evaluate the therapeutic means of
in combination with the measurement of \( p_t \text{O}_2 \) has not been investigated in this context to date. Furthermore, most investigators performed their studies on dog models. Our TEM findings in pigs with acute ischemia and without TMLR were similar to those of Jennings and coworkers [19], who already described the same myocardial structural changes after acute ischemia in 1974. Their results were later confirmed by other authors [20–22]. Jennings investigations were performed on dogs, however, he described in his work the state of reversible and irreversible myocardial cell structural damage. Reversible structural changes establishes itself as a light swelling of the mitochondria, a dilatation of the sarcoplasmic reticulum and a clumping of the chromatin in the cell nucleus. Irreversible cell structural changes show a significant swelling of the mitochondria with ruptured cristae, a damaged matrix with the appearance of amorphic thickenings, as well as a clear margination and clamping of the cell nucleus chromatins. We found, after 30 min of ischemia, the same signs of irreversible myocardial damage and these ultrastructural changes were even clearer 6 h after induction of ischemia. We observed structural damage in terms of clamping of the cell nucleus chromatins, damage of the mitochondria by rupturing of the cristae as well as the appearance of amorphic structures. Furthermore, we detected damage to the myofibrils which vanished and ruptured in the area of the Z-stripes and moreover, we could describe in the course of the tissue analysis, the appearance of lipid drops that indicated cell ischemia.

The findings of Wittaker and colleagues [9], who applied the TMLR 30 min after ischemic induction in a dog model and detected no protective effect, is confirmed by our results in as much as we found irreversible changes in the myocardial ultrastructure at 30 min of ischemia in TEM biopsies. To conclude, in an acute ischemic pig model, TMLR performed not later than 9 min after induction of ischemia has no therapeutic effect on ultrastructural cell changes and could not increase \( p_t \text{O}_2 \). It still remains to be determined how far these results can be transferred to the clinical situation with chronic ischemia and excessive collateralization of the coronary vessels. Further investigations have to prove short- and long-term effects of TMLR on chronic ischemic myocardium.

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References


Appendix A. Conference discussion

Dr J. Vaage (Stockholm, Sweden): What was your hypothesis in this? Why do you think that the TMR should help this acute ischemia? Because this is a situation completely different from when TMR is used clinically. Did you expect that one hole per cm² will reperfuse the ischemic myocardium.

Dr M. Misfeld (Lubeck, Germany): Well, the exact mechanism of action is still unknown. There seems to be some evidence that these channels may remain open and there is a perfusion by these channels from the left ventricular chamber. And we more or less tried to prove this by electron-microscope findings. But you are right when you say that our model is difficult to translate into the clinical situation of the patients, because we have a chronic ischemic situation in the patients. This acute ischemic model of pigs is not a good model to show the effects of TMLR. So we have to use a chronic ischemic model of pigs, not the acute one.

Dr J. Vaage: How did you try to qualify the EM changes? Because just looking at the EM pictures you can basically find whatever you want. You didn’t try any morphometric analysis.

Dr M. Misfeld: No, we just compared our findings to those of Jennings, who described, in a dog model ultrastructural changes in acute ischemia. We were not able to determine the ultrastructural changes time related, so we could not say that after 15 min these changes have not occurred and after 30 min they are full expressed. There are different stages of these changes, but after 30 min and 6 h they are almost the same.